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Comparative study of the inclusion complexation of uracil and 5-fluorouracil with native and modified cyclodextrins: some theoretical and practical considerations

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In this study, the inclusion complexes of α-, β-cyclodextrins and derivatives hydroxypropyl-α-, hydroxypropyl-β-cyclodextrins with uracil and the anti-cancer agent 5-fluorouracil were demonstrated by UV-Vis spectroscopy and quantum chemical calculations. The complexes stability constants and the thermodynamic parameters for the 1:1 stoichiometry inclusion complexes were obtained and compared. The thermodynamic analysis of the studied complexes showed that the inclusion reaction is an exothermic spontaneous reaction and is an enthalpy driven process for the temperature domain of 298K to 313K. Theoretical calculations were performed on complexes to examine the energetic quantities involved in the stability of the complexes. The correlation of the energy parameters obtained from experimental and theoretical data suggests a high affinity between cyclodextrins and both uracil and 5-fluorouracil molecules.

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Keywords: Cyclodextrin, Uracil, 5-fluorouracil, Inclusion complexes, Thermal behavior

1. Introduction

Uracil (U) and 5-fluorouracil (*5-Fluoro-1H,3H-pyrimidine-2,4-dione*, 5FU) are two closely related compounds that differ in their chemical structure and properties. The interaction between cyclodextrins (CDs) with U or 5FU is of great importance due to its potential applications in various fields, such as pharmaceuticals and drug delivery systems. These complexes have shown promising results in improving the solubility, stability, and therapeutic efficacy of uracil-based drugs.

CDs are a family of cyclic oligosaccharides composed of a macrocyclic ring of glucose subunits joined by α-1,4 glycosidic bonds [1]. They are formed from starch through enzymatic conversion and have various applications in the food, pharmaceutical, drug delivery, chemical, agriculture, and environmental engineering industries [1]. The structures of CDs are toroidal, with a larger and a smaller opening of the toroid exposing primary and secondary hydroxyl groups, respectively. The interior of the CD toroid is hydrophobic, while the exterior is hydrophilic [1]. This unique structure allows CD to form inclusion complexes with hydrophobic compounds, enhancing their solubility [2, 3, 4]. 2-hydroxypropylated-CDs have been generally recognized as safe in pharmaceutical applications with low toxicity [2]. The structure of 2-hydroxypropylated-CD involves the attachment of hydroxypropyl groups to the hydroxyl groups of CD. This modification increases the lipophilicity of CD and enhances its ability to form inclusion complexes with hydrophobic molecules [2]. The degree of substitution can be influenced by varying the alkaline concentration in the synthesis phase, resulting in different hydrophobicities and properties of the modified CD [2, 5, 6, 7]**.** The guest molecules, U and 5FU are two pyrimidine derivative compounds that exhibit weak acidity behavior and exhibit light-dependent properties [8]. Uracil is one of the four nucleobases found in the nucleic acid RNA and it has the ability to undergo amide-imidic acid tautomeric shifts, allowing it to maintain stability [9, 10]. It binds to adenine via two

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hydrogen bonds in RNA, while in DNA, uracil is rarely found and is believed to have been replaced by thymine during evolution to increase genetic stability. [11, 12]. 5FU is a synthetic analog of U that is commonly used as an anticancer drug. Due to the presence of the fluorine atom, 5FU exhibits different pharmacokinetic properties and improved antitumor activity compared to U. It is widely used in chemotherapy regimens to treat various types of cancer [11, 12]. 5FU exists significantly as an ionized tautomer that can mispair with guanine during DNA replication [13]. Its importance lies in its ability to inhibit thymidylate synthase, an enzyme involved in DNA replication. By preventing the production of deoxythymidine monophosphate, a building block for DNA synthesis, 5FU inhibits cell growth and induces cell death [14]. Additionally, 5FU can be incorporated into RNA, leading to RNA damage and disruption of cellular functions [14]. Both U and 5FU have been extensively studied for their physicochemical properties, and further research is continuously being conducted to explore their potential uses in various fields, including drug discovery and the development of new therapeutic agents.

In the field of gene therapy, which aims to treat diseases by introducing genetic material into cells, CDs have been used as carriers for nucleic acids, including RNA. CDs can form inclusion complexes with RNA, protecting it from degradation and facilitating its delivery into cells [15]. By interacting with RNA nucleobases, CDs can enhance the stability and efficiency of gene delivery systems, improving the therapeutic potential of RNA-based therapies.

The formation of inclusion complexes between CDs and uracil or its derivatives has been studied and reported in the literature. One study investigated the complexation between 5FU, a pyrimidine analog used as a chemotherapeutic agent, and βCD [15]. This article examines the complex formation between 5FU and βCD using various spectroscopic techniques, including NMR and IR spectroscopy. This study provides important insights into the potential applications of βCD/5FU complexes in cancer chemotherapy [16]. The influence of different factors on the release kinetics of 5FU from the complexes and valuable insights into the potential applications of CD complexes in drug delivery systems were provided in other important work [17]. Another study explored the complexation between 5FU and both αCD and βCD [18]. The inclusion complexes were prepared in the solid state using the kneading method and characterized by X-ray powder diffractometry and FT-IR spectroscopy. The study showed that the formation of the inclusion complexes improved the bioavailability and solubility of 5FU. In addition, the cytotoxic activity of 5FU complexed with βCD was found to be higher against breast cancer cells, while the complexation with αCD exhibited higher cytotoxic activity against alveolar basal epithelial carcinoma cells [18]. Also, the solid state complexes between CDs and U and 5FU were prepared in the solid state using the melting in solution method and investigated through various techniques for understanding the thermal behavior, molecular structure, and morphology [19]. Another study focuses on the inclusion complexes of lumichrome and lumazine with βCD. The authors investigate the formation and properties of these complexes using various experimental techniques, including NMR spectroscopy. The study provides valuable information about the complexation behavior and potential applications of lumichrome and lumazine in the pharmaceutical field [20].

Along with experimental investigations, molecular dynamics simulations and other theoretical studies were conducted to analyze the interactions and behavior, especially for the complex between 5FU and βCD [16, 18]. An important study by DFT (Density-functional theory) considering the effect of dispersion interaction for both inner and outer arrangements of host-guest molecules (5FU and βCD) was done in the gas phase and in water, modeled by the polarizable continuum model of solvation [21]. Also, investigates of the complex formation between βCD and the anticancer drug 5FU using molecular dynamics simulations was accomplished for the determination of the thermodynamic parameters and interaction energies associated with the complexation process considering initial trial geometries both in a 1:1 and 1:2 stoichiometry [22].

While there have been previous studies on the characterization of βCD inclusion complexes with U and 5FU, both from experimental and computational perspectives, the authors believe that further research is needed to fully understand the complex formation process. To gain a deeper understanding of the physical mechanisms that elucidate the formation of solid-state inclusion compounds between host molecules αCD, βCD, HPαCD, HPβCD, and guests U or 5FU,

our study utilized quantum chemistry calculations and UV-Vis spectroscopy data. The following aspects differentiate this work from previously investigated similar systems:

- Thermodynamic parameters of interaction obtained by the UV-Vis spectroscopy method for each complex formed between considered CDs and guests U and 5FU;
- Quantum chemical calculations were accomplished for different start configurations of the Host-Guest assemblies and various energies involved in the description of the assemblies were determined.

The findings presented in this paper contribute to clarifying the relationship between the thermodynamic stability of the complexes, the structural factors involved, and the efficiency of complexation.

2. Materials and methods

2.1. Materials

The uracil (U) - purity 99% was purchased from Loba Chemie, 5-fluorouracil (5FU) - purity 99%, α-Cyclodextrin (αCD) - purity 98%, β-Cyclodextrin (βCD) - purity 97%, (2-Hydroxypropyl)-α-Cyclodextrin (HPαCD), (2-Hydroxypropyl)-β-Cyclodextrin (HPβCD) were purchased from Sigma Aldrich Chemical Company. All substances were used without further purification.

2.2. Preparation of the samples

The solid state inclusion compound CD/Guest was prepared in a 1:1 molar ratio of the CD (each of the αCD, βCD, HPαCD, HPβCD) and U or 5FU using „melting in solution" method, procedure previously reported in the literature [23]. The solid powder of CD was dissolved in double distilled water (10^{-5} mol/L) then the corresponding quantity of solid guest (U or 5FU) was added in reaction. The solution mixture was firstly sonicated for 5 min. at 45 kHz, and then stirred (600 rpm) for 24h at room temperature. The CD/Guest solid powder was obtained after dehydration under vacuum at 60°C. In UV-Vis experiments, concentrations of 10^{-5} mol/L for guest U/5FU and of 0 to 2 x 10^{-5} mol/L for CDs aqueous solutions were used in order to avoid a predominance of the dimer over the monomer.

2.3. Methods

2.3.1. Ultraviolet-Visible (UV-Vis) Spectroscopy

All records of UV-Vis spectra were carried out on a Carry 300 Bio spectrophotometer equipped with a temperature-controlled cell holder using 1x1x4 - cm micro quartz cells with teflon stopper. The stoichiometry of the inclusion complexes was assessed by continuous variation method (Job's method) by varying the mole fraction of each component (R = [guest] /([guest] + [host])) from 0 to 1 and the total molar concentration of the species is kept constant (10^{-6} M). After 24 h the absorption spectra were recorded at 25ºC. The difference in absorbance (A) measured at 258 nm for U and 265 nm for 5FU, respectively, between solutions containing only guest and the CD/Guest mixtures, multiplied by the molar ratio (R) of guest was plotted as a function of the R of the guest [24, 25]. The stoichiometric ratio of the inclusion complex is corresponding to the point where the derivative of the curve is zero. The Job's plots of the analyzed systems (αCD/U, βCD/U, HPαCD/U, HPβHP/U, αCD/5FU, βCD/5FU, HPαCD/5FU, HPβCD/5FU) were presented in Figure 4 a), b). The quantitative determination of the apparent stability constant (K) of the inclusion complexes was done spectrophotometrically at 4 different temperatures (298.15 K, 303.15 K, 308.15 K, 313.15 K). To obtain the K values the guest concentration was kept constant (10^{-6} M) and the CD concentration was varied from 0 to 200 mol/Kg. The CD solutions of corresponding concentrations were used in the reference cuvette [26, 27].

2.3.2. Computational details

The quantum chemical calculations were performed using the facilities of the GAMESS package program [28] installed on a 65 blades IBM cluster. The geometries of the studied systems were initially optimized by Hartree-Fock (HF) calculations, using gradually better and better wave

functions sets: STO-3G, STO-6G, n311-6G, and finally valence triple zeta (TZV) [29]. To evidence the effect of the dispersion corrections, further DFT B97-D/6-31G (d, p) calculations were performed [28].

In the HF calculations, the optimization of the H - G complex starts with the configuration of the assembly containing the optimized geometries of the H and G molecules. The final configurations obtained by HF method were used as the start configurations to perform the DFT calculations, so the geometries of the isolated constituent molecules are also optimized by the DFT method. Anyways, there are infinite such configurations and it is enough to choose some of them and test each time if the inclusion complex was finally obtained. Consequently, three different start configurations of the H-G assemblies were considered and named according to the start configuration used in calculations: H-G-L, H-G-M and H-G-R. The G molecule (5FU and U) is perpendicular on the Oz axis and located either at the entrance of the large side of H (Oz = +3.6 Å), in the middle of the cavity (Oz = 0), and at the entrance of the narrow side of the H (Oz = - 3.6 Å). For instance, Figure 1 shows the inclusion complex named CD-5FU-R, formed by 5FU molecule with CD when in the start configuration is located on the right side of CD (large side of the CD's cavity), at Oz = +3.6 Å.

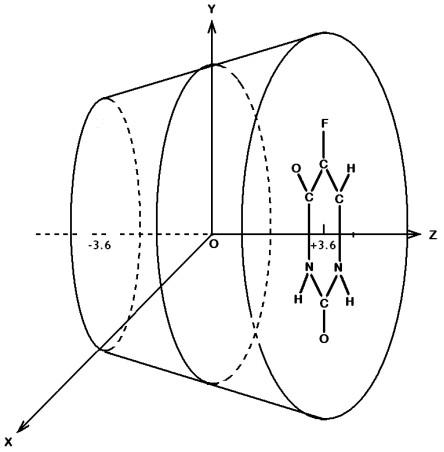


Fig 1. The coordinate system for the 1:1 5FU complexation reaction with CD in start configuration CD-5FU-R.

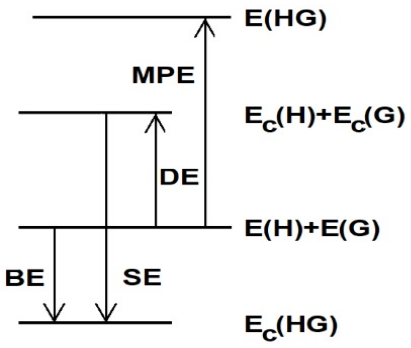
In order to evaluate the H-G interactions the values of the following quantities are computed [16]:

1) Binding energy, *BE*, is the difference between the energy of the inclusion complex and the sum of the energies of the component molecules.

2) Deformation energy, *DE*, is the difference between the energy of the isolated molecule with optimized geometry and the molecule energy that has the geometry from the inclusion complex.

3) Mutual perturbation energy, *MPE or –SE (SE is the stabilization energy)* is the difference between the assembly energy and the sum of the energies of the two isolated molecules.

The *BE, DE, MPE* and *SE* quantities are computed both by HF and DFT methods. The mathematical form and the involved energies in the calculation of the *MPE, SE, BE* and *DE* are depicted in Figure 2.



$$MPE(HG) = E(HG) - E(H) - E(G)$$
$$DE(HG) = E(HG) - E_c(HG)$$
$$BE(HG) = E_c(HG) - E(H) - E(G)$$
$$SE = E_c(HG) - E_c(H) - E_c(G) = BE - DE$$
$$SE = - MPE$$

Fig. 2 The various energies involved in computing of the MPE, SE, BE, DE quantities: E(HG) – the energy of the host – guest assembly, H and G molecules having optimized geometries; $E_c(HG)$ – the energy of the inclusion complex; E(H) + E(G) – the sum of the energies of the optimized geometries of the host and guest molecules, $E_c(H) + E_c(G)$ – the sum of the energies of the H and G molecules. The "c" index refer to molecules or assemblies which have the geometry from the complex. The energies without the "c" index refer to molecules that have optimized geometries.

3. Results and discussion

3.1. UV-Vis spectrometry

UV-Vis spectroscopic studies of the host-guest interactions were done to establish the stoichiometry and the stability constant of the different CDs and U or 5FU complexes. Also, thermodynamic parameters of the complex formation were obtained from UV-Vis resulted data. In Figure 3 the spectra of absorption maximum wavelengths observed at 258 nm for U and 265 nm for 5FU, respectively in the presence of different CD types are presented.

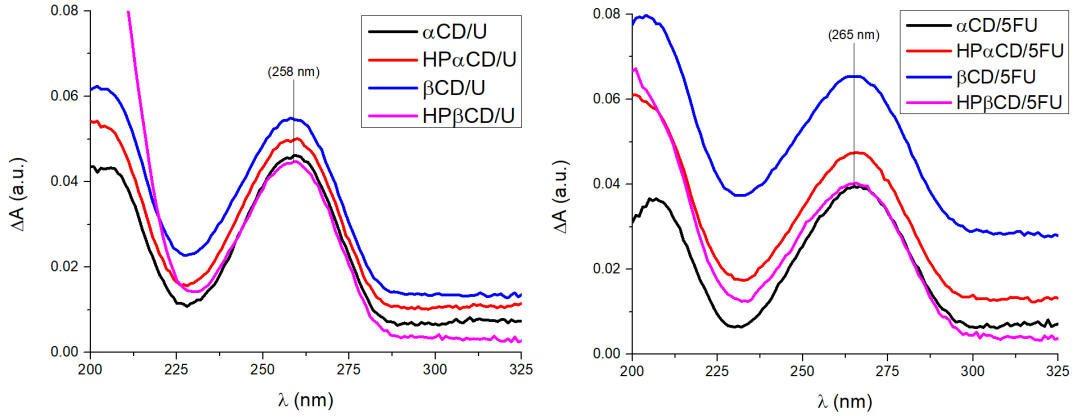


Fig. 3. Variation of absorbance for the complexes formed between U and 5FU in presence of different aqueous CDs solutions at 298.15 K.

The spectra were recorded in pure water at a temperature of 298.15 ± 0.5 K, p = 0.1 MPa, and at the same concentration for each of the used CDs. It can be observed that on a very narrow absorbance domain the spectra of U and 5FU are more affected in the presence of HPβCD than in presence of αCD. Interaction with the CDs and other biological molecules depends on the form (ionized or neutral) of U or 5FU. Also, the ability of such molecules to interact with CDs is strongly dependent on the solvent type and temperature. The absorption maximum wavelengths observed at 258 nm for U and 265 nm for 5FU, respectively are specific for a neutral pH and this corresponds to their specific tautomeric forms for a neutral aqueous solution environment [30, 31].

Further, the method of continuous variation (Job's plot), was used to determine the stoichiometry of the inclusion complexes [24, 25].

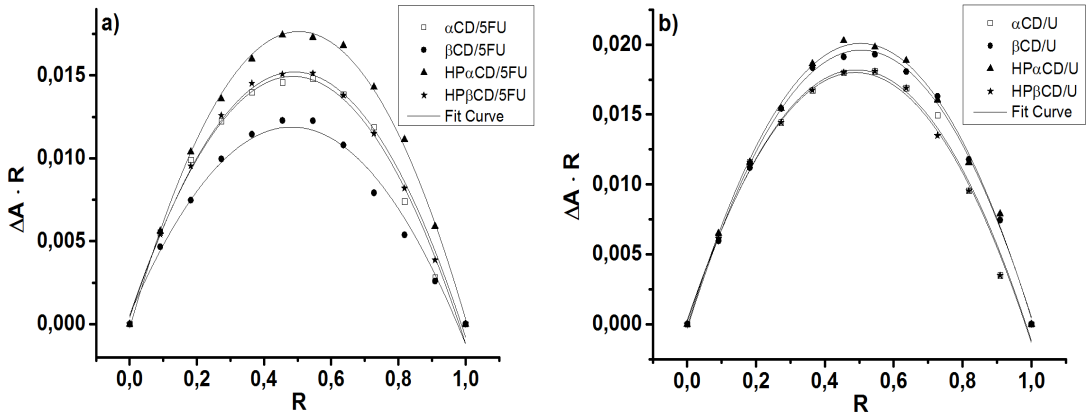


Fig. 4. Continuous variation plots (Job's plots) of CDs with a) – U and b) – 5FU.

The curves presented in Figure 4 have a maximum at R = 0.5 indicating a predominant 1:1 stoichiometric ratio for each of the complex between the U/5FU and CDs. Considering the narrow temperature range (298K to 313K) for which the thermodynamic properties of these complexes were investigated, it can be observed (in Table 1) that the obtained values of the apparent association constants decrease with increasing temperature, as expected for an exothermic process. The resulting values of binding constants show that the guest molecule (U or 5FU) has a slight preference for hydroxypropylated cyclodextrins. The complexes HPβCD/5FU and HPβCD/U have the highest constants at 298K but with differences in the temperature range due to the fact that the HPβCD is affected by the thermal factor, it produces deformations in HPβCD, but U and 5FU still remain deeply included into the CD cavity. The lowest values of binding constant were observed for the complexes obtained with HPαCD and αCD. Along with the increase in temperature, the values of ΔG became slightly less negative, implying that higher temperature could destabilize the complexes [32]. This can be caused by hydrogen bonds which usually are weakened by heating but also by the solubilization of the guest molecule [33]. The literature data present the apparent association constants for the association of αCD and βCD with 5FU through absorbance measurements at different pH values. It was reported that the values of the constants are 74 and 563 for the 5-FU:alpha-cyclodextrin while for the 5-FU:beta-cyclodextrin complex between 187 and 559, in good agreement with other published values [9, 16, 18]. In this work, the resulting binding constants values seem to be consistent with those previously communicated in literature.

Table 1. Values of the equilibrium constant K, of 1:1 complex formation for U/5FU with CDs at different temperatures.

***T* (K)**	***K* (L/mol)**							
	αCD/U	αCD/5FU	HPαCD/U	HPαCD/5FU	βCD/U	βCD/5FU	HPβCD/U	HPβCD/5FU
298.15	47.1	125.7	133.8	172.5	71.5	155.3	177.6	218.2
303.15	43.3	111.2	129.2	146.8	69.7	132.7	143.5	186.3
308.15	42.3	92.8	93.4	112.6	51.0	109.9	102.9	162.7
313.15	38.6	88.2	77.2	95.4	53.7	92.6	95.8	102.4

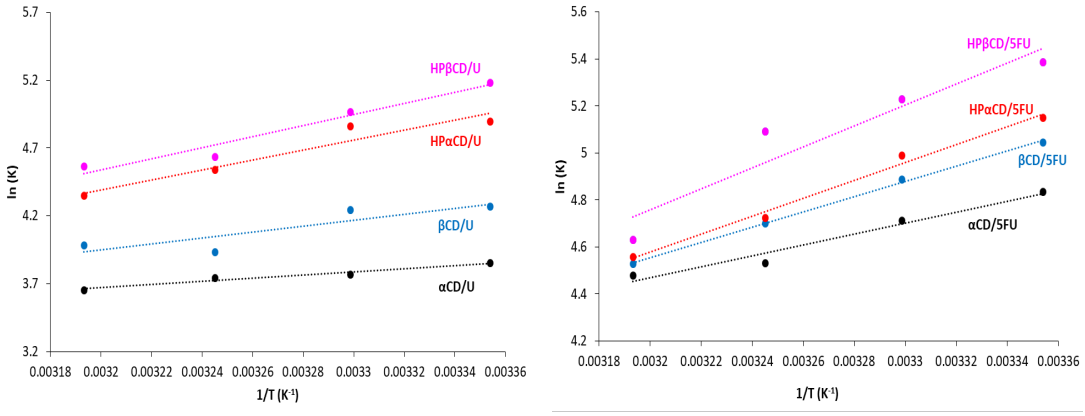


Fig. 5. Van't Hoff plots for complexes between U and CDs (left panel) and for complexes between 5FU and CDs (right panel).

Table 2. Values of thermodynamic parameters (the standard molar enthalpy of binding ΔH^0, the standard Gibbs free energy change ΔG^0 and the standard entropy change ΔS^0) of 1:1 complex formation of U/5FU with CDs.

Sample	ΔG^0_{298} (kcal/mol)	ΔH^0 (kcal/mol)	ΔS^0 (cal/mol·K)
αCD/U	-2.28	-2.29	-0.1
βCD/U	-2.54	-4.35	-6.08
HPαCD/U	-2.94	-7.30	-14.64
HPβCD/U	-3.06	-8.12	-16.95
αCD/5FU	-2.83	-4.62	-5.91
βCD/5FU	-2.99	-6.45	-11.59
HPαCD/5FU	-3.06	-7.57	-15.14
HPβCD/5FU	-3.22	-8.88	-18.96

The thermodynamic parameters of the complexes derived from UV–vis spectroscopic data were determined from the temperature dependence of the stability constant (Figure 5) using the van't Hoff equation [34, 35]. The resulted values of standard enthalpy and entropy change were used in Gibbs-Helmholtz equation to calculate the free energy variation (ΔG^0) and the obtained values are reported in Table 2. As can be observed, for all investigated systems the value of ΔG^0 was found negative suggesting that the complexation process occurred spontaneously, whereas the negative value of ΔH^0 and small negative value of ΔS^0 indicate that the inclusion process is exothermic and enthalpy favored. The small negative entropy change is caused by the contribution of the loss of translational and rotational degrees of freedom of U and 5FU in the cavity of CDs that exceeds the contribution of the water rearrangement/displacement upon complexation [36]. The orientation or motion of guest molecule in the CD cavity can be controlled by electrostatic interactions and by hydrogen bonding [36]. Furthermore, the studied complexes having the negative ΔG^0 values, imply that the Gibbs energy change becomes more negative as the cavity diameter of CD increases, and the inclusion process with HPβCD becomes more favored despite the difference in ΔS^0 values.

3.2. Computational results

Whatever the start configuration, stable inclusion configurations are obtained for any of the H-G considered systems. It was observed that the guest molecule was not expelled from the CD's cavity and the energy of the final configuration is lower than the sum of energies of the constituent molecules with optimized geometries. The obtained results have shown two different situations.

i) When the guest molecule is located in the right side of CD for start configuration and after optimization there are formed several hydrogen bonds between H and G molecules (Figures 6 and 7); these bonds have fixed the G (U or 5FU) molecule plane perpendicular to Oz axis. Figures 6 and 7 show the front view of the final configurations of the H-U-R and H-5FU-R of the 1:1 inclusion complexes.

ii) When the guest molecule is located either in the middle or on the left side of the CD there are established weak interactions. For –L type complexes the G molecule is only rotated, its plane becoming almost parallel to the Oz axis (Figure 8). In -L and -M complexes none of the G molecules is moved along the Oz axis.

The corresponding values of calculated energies obtained by HF are shown in Tables 3 and 4. MOLDEN software was used for the structure visualization [37].

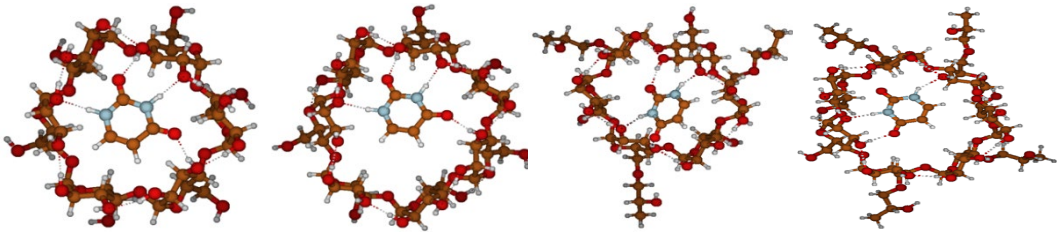


Fig. 6 The final configurations of the H-U-R type inclusion complexes: a) αCD-U-R, b) βCD-U-R, c) HPαCD-U-R, d) HPβCD-U-R.

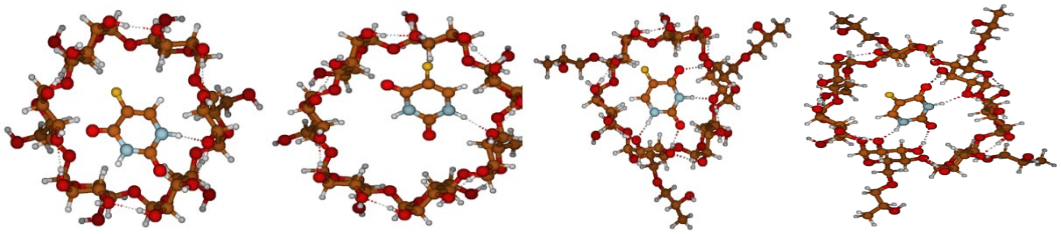


Fig. 7. The final configurations of the H-5FU-R type inclusion complexes: a) αCD-5FU-R, b) βCD-5FU-R, c) HPαCD-5FU-R, d) HPβCD-5FU-R.

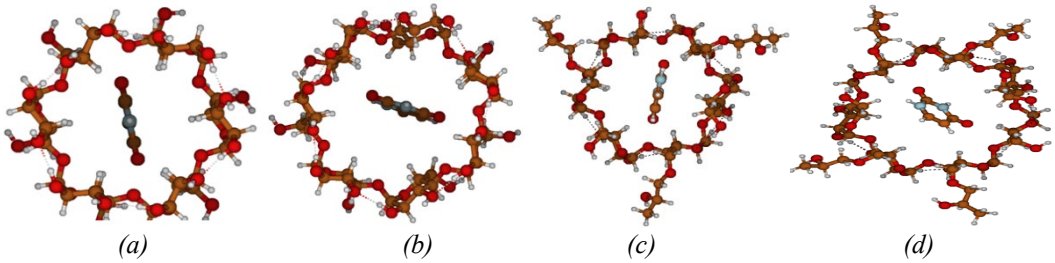
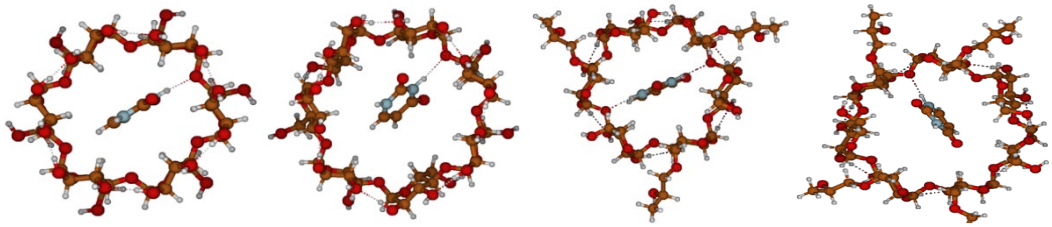


Fig. 8 The final configurations of the H-U-L type inclusion complexes: a) αCD-U-L, b) βCD-U-L, c) HPαCD-U-L, d) HPβCD-U-L.



(a) (b) (c) (d)

Fig. 9. The final configurations of the H-U-M type inclusion complexes: a) αCD-U-M, b) βCD-U-M, c) HPαCD-U-M, d) HPβCD-U-M.

Table 3. The calculated values of the MPE(HG), DE(HG), BE(HG), SE(HG) energy types for inclusion complexes between host CDs molecules with -L, -M, -R position of 5FU. The calculations are accomplished by using HF method and the obtained values are expressed in kcal/mol.

Computed energy	**Cyclodextrin Type**				
	Complex Type	**αCD**	**βCD**	**HPαCD**	**HPβCD**
BE(HG)	**H-U-L**	-7.348	-5.374	-6.387	-7.079
	H-U-M	-3.811	-8.051	-10.992	-11.034
	H-U-R	-16.709	-25.994	-15.302	-19.607
DE(HG)	**H-U-L**	0.511	2.953	0.451	0.615
	H-U-M	2.232	1.464	2.713	1.839
	H-U-R	18.320	9.497	18.320	15.033
MPE(HG)	**H-U-L**	7.859	8.327	6.928	7.694
	H-U-M	6.044	9.516	13.705	12.423
	H-U-R	35.029	35.491	33.622	34.640
SE(HG)	**H-U-L**	-7.859	-8.327	-6.834	-7.694
	H-U-M	-6.043	-9.515	-13.705	-12.423
	H-U-R	-35.029	-35.491	-33.622	-34.640

Table 4. The calculated values of the MPE(HG), DE(HG), BE(HG), SE(HG) energy types for inclusion complexes between host CDs molecules with -L, -M, -R position of 5FU. The calculations are accomplished by using HF method and the obtained values are expressed in kcal/mol.

Computed energy	**Cyclodextrin Type**				
	Complex Type	**αCD**	**βCD**	**HPαCD**	**HPβCD**
BE(HG)	**H-5FU-L**	-6.065	-2.699	-6.387	-6.199
	H-5FU-M	-6.632	-11.063	-10.281	-9.645
	H-5FU-R	-15.575	-7.572	-21.899	-22.693
DE(HG)	**H-5FU-L**	0.554	5.132	0.114	0.615
	H-5FU-M	2.354	1.909	3.622	1.839
	H-5FU-R	8.915	4.209	5.524	15.033
MPE(HG)	**H-5FU-L**	6.619	7.832	6.865	7.694
	H-5FU-M	8.985	12.973	13.903	12.423
	H-5FU-R	19.639	11.781	27.423	34.640
SE(HG)	**H-5FU-L**	-6.619	-7.831	-6.834	-6.814
	H-5FU-M	-8.986	-12.972	-13.705	-11.484
	H-5FU-R	-24.490	-11.781	-27.423	-37.726

Without the dispersion correction, the energy values have shown that the intermolecular interactions remain weak. The obtained results indicate that intermolecular interactions are more significant in the -R type complexes than in the -L or -M type. Considering the results obtained by the HF method, all analyzed assemblies have formed inclusion complexes; even in the absence of

the London dispersion none of the guest molecules is expelled out of the CD's cavity [38]. Therefore, this observation was confirmed by DFT calculations.

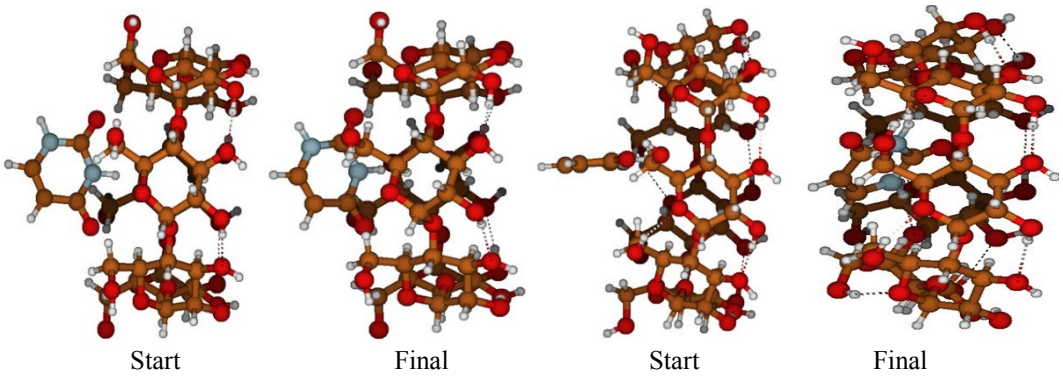


Fig. 10 The start and final configurations of the CD-U-L complexes: a) αCD-U-L, b) βCD-U-L

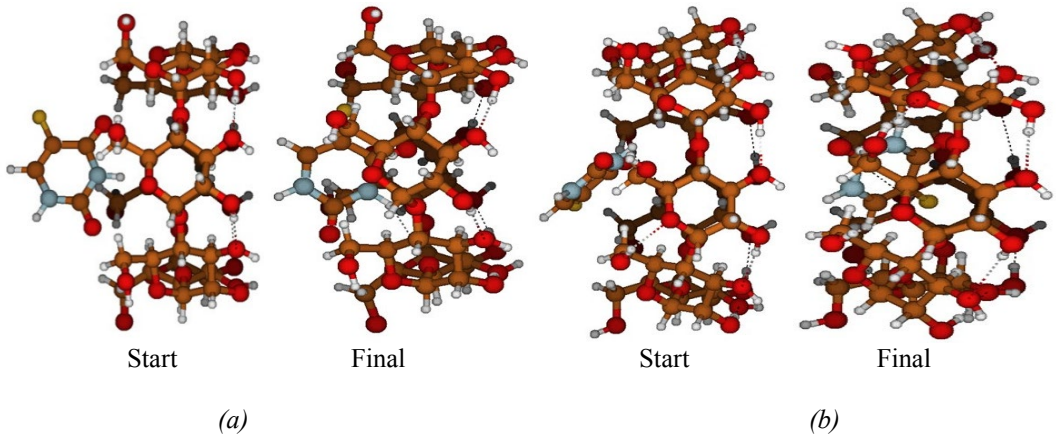


Fig. 11. The start and final configurations of the CD-5FU-L complexes: a) αCD, b) βCD.

Table 5. The calculated values of the MPE(HG), DE(HG), BE(HG), SE(HG) energies for inclusion complexes between host CDs molecules with -L, -M, -R position of U. The calculations are accomplished by using DFT B97-D/6-31G (d, p) and the obtained values are expressed in kcal/mol

Computed energy	**Cyclodextrin Type**				
	Complex type	**αCD**	**βCD**	**HPαCD**	**HPβCD**
BE(HG)	**H-U-L**	-17.30	-29.80	-20.30	-37.10
	H-U-M	-29.10	-28.60	-34.80	-36.70
	H-U-R	-38.20	-36.90	-40.80	-52.50
DE(HG)	**H-U-L**	2.84	3.92	1.91	3.19
	H-U-M	1.82	9.15	7.74	0.22
	H-U-R	24.30	22.1	19.6	18.30
MPE(HG)	**H-U-L**	20.13	33.73	22.22	40.29
	H-U-M	30.91	37.77	42.56	36.91
	H-U-R	62.52	59.01	64.41	71.82
SE(HG)	**H-U-L**	-20.14	-33.72	-22.21	-40.29
	H-U-M	-30.92	-37.75	-42.54	-36.92
	H-U-R	-62.50	-59.00	-64.40	-70.80

Table 6. The calculated values of the MPE(HG), DE(HG), BE(HG), SE(HG) energy types for inclusion complexes between host CDs molecules with -L, -M, -R position of 5FU. The calculations are accomplished by using DFT B97-D/6-31G (d, p) and the obtained values are expressed in kcal/mol.

Computed energy	**Cyclodextrin Type**				
	Complex type	**αCD**	**βCD**	**HPαCD**	**HPβCD**
BE(HG)	**H-5FU-L**	-9.44	-22.90	-24.60	-35.2
	H-5FU-M	-31.80	-28.90	-29.00	-32.2
	H-5FU-R	-31.30	-29.70	-34.4	-50.8
DE(HG)	**H-5FU-L**	3.97	4.06	7.16	0.91
	H-5FU-M	2.11	3.44	8.61	3.21
	H-5FU-R	11.1	9.71	19.0	17.1
MPE(HG)	**H-5FU-L**	13.41	26.96	31.76	36.12
	H-5FU-M	33.90	32.33	37.63	35.41
	H-5FU-R	42.39	39.41	53.41	67.88
SE(HG)	**H-5FU-L**	-13.41	-26.96	-31.76	-36.11
	H-5FU-M	-33.91	-32.34	-37.61	-35.41
	H-5FU-R	-42.40	-39.41	-53.40	-67.90

After performing DFT calculations in the –L type configurations the U and 5FU molecules are included almost completely in the CD cavity, the resulted –L type configurations becoming similar to those of -M type complexes. The geometrical changes obtained for –L complexes are exemplified for αCD-U/5FU-L and βCD U/5FU-L are shown in Figures 10 and 11. It was observed that in the resulted –R type complexes the constituent molecules are interconnected by hydrogen bridges. These bonds are strengthened after performing DFT calculations, consequently, the stability was increased too. The corresponding energy values are done in Tables 5 and 6.

The results obtained by DFT calculations (Tables 5 and 6) show that the BE values are lower for -L complexes than for –R complexes. The values of DE for complexes obtained between CD with U guest are lower than for CD-5FU complexes. The large values of DE are caused by choosing the relative position of the two constituent molecules, in order to obtain maximized values of BE. Therefore, in CD-U complexes, both U guest and CD molecule has to be deformed to maximize the BE.

4. Conclusions

In this work UV-Vis spectroscopy measurements and quantum chemical calculations we accomplished to evaluate the behavior of pyrimidine derivatives U and 5FU in the presence of different CD types. It was established that the four cyclodextrins (αCD, βCD, HPαCD, HPβCD) can form stable complexes with both U and 5FU at different temperatures in the domain of 298K to 313K. The stoichiometry for all the complexes was demonstrated to be a predominant 1:1 ratio. It was revealed that the obtained apparent association constants decrease with increasing temperature and it was established that the stability of the complexes at the temperature of 298K is as follows: HPβCD/5FU > HPβCD/U > HPαCD/5FU > βCD/5FU > HPαCD/U > αCD/5FU > βCD/U > αCD/U. The thermodynamic analysis of the studied complexes showed that the inclusion reaction is an exothermic spontaneous reaction and is an enthalpy driven process. By theoretical calculations were calculated the energetic quantities of three types of inclusion configurations. It was shown that each geometry of the complex has the guest molecule inside the cavity of CDs and the systems containing HPβCD are more interconnected through hydrogen bridges and more energetically stable. The complexes established between CDs and 5FU present higher negative values for energy types than complexes between CDs and U. The correlation of the energy parameters obtained from experimental and theoretical data suggests a good affinity between cyclodextrins and both uracil and 5-fluorouracil molecules. The most stable complexes are formed

between HPβCD and guest molecules and it could be concluded that all the analyzed complexes are stable in the physiological temperature domain.

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